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Evaluation of Latin-American fruits rich in phytochemicals with biological effects

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ABSTRACT

This work aimed to provide a thorough description of the polyphenolic composition of five Latin-American fruits of increasing interest, which have certain anti-diabetic effects (açaí, maqui, Cape gooseberry, papaya and noni), and to correlate their antioxidant capacity and anti-diabesity activities (lipase and α-glucosidase inhibition), and examine their potential use by the food industry. The phytochemical profiling of the fruits revealed a wide range of bioactive phenolics. The inhibition of pancreatic lipase was significant for maqui, and maqui and papaya were the best inhibitors of α-glucosidase. Regarding the DPPH, $ABTS^{\cdot+}$ and FRAP assays, maqui berries displayed the highest activity. The ORAC method and the superoxide radical scavenging assays revealed maqui and açaí as the best performers. These Latin-American fruits are of great value regarding nutrition and health benefits, and the development of products for the control of diabetes and obesity.

1. Introduction

In the last decade, numerous publications have dealt with the high content of bioactive compounds, mostly polyphenols, present in certain fruits and their protective effects on human health. Besides their antioxidant properties, it is widely accepted that most of these phenolics have an affinity for proteins, exhibiting inhibitory activity on some functional enzymes (Birari & Bhutani, 2007). In this aspect, the inhibition of α-glucosidase, a key enzyme that catalyzes the final step in the digestive process of carbohydrates, could delay the breakdown of oligosaccharides and disaccharides into monosaccharides, diminishing glucose absorption and consequently reducing postprandial hyperglycaemia (Rubilar et al., 2011). Berry polyphenols have been reported as being inhibitors of α-glucosidase *in vitro* (Boath, Stewart, & McDougall, 2012). The current therapeutic approaches for the treatment of obesity involve the inhibition of dietary triacylglycerol absorption, via inhibition of pancreatic lipase (PL) by orlistat (Birari & Bhutani, 2007). Many polyphenolic extracts are active against this enzyme; for example, polyphenol-rich water extracts from litchi (*Litchi chinensis* Sonn.) show *in vitro* inhibitory effects (Wu et al., 2013), while extracts from certain berries have been described as effective inhibitors of PL *in vivo* (McDougall, Kulkarni, & Stewart, 2009).

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The increase in human obesity has been accompanied by a growing incidence of diabetes. The close relationship between these two conditions has led to the adoption of the term *diabesity* (Schröder, 2007). In this sense, some fruits of Latin-American origin represent good sources of bioactive compounds with certain anti-diabetic effects and are receiving increasing interest. Açaí (*Euterpe oleracea* L.) is a palm-tree berry from the Amazon area in South America. Potential benefits have been attributed to açaí fruits, extracts and juices: antioxidant, anti-inflammatory (Schauss et al., 2006), hypocholesterolaemic (De Souza et al., 2012) and anti-diabetic activity (Kim, Hong, Jung, Jeong, & Cho, 2012). Maqui (*Aristotelia chilensis* L.) is a common edible berry from central and southern Chile, and it is a source of natural colorant due to the presence of anthocyanins. Various reports have linked the phenolics of maqui berries with their high antioxidant capacity (Rubilar et al., 2011), *in vitro* inhibition of adipogenesis and inflammation (Schreckinger, Wang, Yousef, Lila, & De Mejia, 2010), cardioprotection (Céspedes, El-Hafidi, Pavon, & Alarcon, 2008) and *in vitro* and *in vivo* anti-diabetic effects (Rojo et al., 2011; Rubilar et al., 2011). Cape gooseberry (*Physalis peruviana* L.) is an herbaceous perennial semi-shrub that grows in sub-tropical zones. Its calyx represents an essential source of carbohydrates during the first 20 days of growth and development. Anti-inflamatory, hypocholesteroleamic and antihepatotoxic effects have been attributed to *P. peruviana* (Ramadan, 2012). Papaya (*Carica papaya* L.) fruits grow in tropical and sub-tropical regions and are marketed around the world. Numerous papers have described beneficial effects of this fruit against chronic diseases such as cancer (Nguyen, Shaw, Parat, & Hewavitharana, 2013), diabetes (Juárez-Rojop et al., 2012) and obesity (Athesh, Karthiga, & Brindha, 2012). Noni (*Morinda citrifolia* L.) is a tropical and sub-tropical plant used as a folk medicine in Pacific islands to treat a broad range of diseases. Recently, several health benefits have been attributed to noni fruits, juice or extracts, namely hypolipidemic and anti-oxidative effects (Lin et al., 2012), hepatoprotection (Wang, Nowicki, Anderson, Jensen, & West, 2008), anti-diabetic (Sabitha, Adhikari Prabha, Shetty Rukmini, Anupama, & Asha, 2009) and anti-cancer (Brown, 2012) effects.

In addition to the above-mentioned bioactivities, it has been reported that these five fruits also display significant anti-diabetic activity (Juárez-Rojop et al., 2012; Kim et al., 2012; Lee et al., 2012; Pujiyanto, Lestari, Suwanto, Budiarti, & Darusman, 2012; Rojo et al., 2011). To the best of our knowledge, there are insufficient data in the literature (arising from the same assaying procedure and conditions) to allow a comprehensive comparison of the different antioxidants and enzymatic activities of these polyphenol-rich fruits. Moreover, the polyphenolic composition has been reported for some of these fruits, mainly açaí and maqui, but phenolic characterization studies on Cape gooseberry, noni and papaya are scarce. Hence, the aim of this study was to evaluate the α-glucosidase- and lipase-inhibitory activities and the antioxidant activities of five fruits rich in bioactive compounds (native from different countries in Latin America), together with their phytochemical profiling, making a comparison of the species, their origin and the analytical methods studied.

2. Material and methods

2.1. *Chemicals*

The compounds 2,2-diphenyl-1-picrylhidracyl radical ($DPPH^{\cdot}$), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)diammonium salt ($ABTS^{\cdot+}$), 2,4,6-tripyridyl-S-triazine (TPTZ), ferric chloride hexahydrate, fluorescein (free acid), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (APPH), monobasic sodium phosphate, dibasic sodium phosphate, Folin Ciocalteu's Reagent, β-nicotinamide adenine dinucleotide (NADH), phenazine methosulphate (PMS), nitrotetrazolium blue chloride (NBT), triazine hydrochloride, 4-nitrophenyl α-D-glucopyranoside, α-glucosidase from *Saccharomyces cerevisiae*, acarbose and potassium phosphate were obtained from Sigma–Aldrich (Steinheim, Germany). Meanwhile, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and magnesium chloride hexahydrate were purchased from Fluka Chemika (Neu-Ulm, Switzerland); sodium carbonate (anhydrous), sodium benzoate, and potassium sorbate were bought from Panreac Química S.A. (Barcelona, Spain). LIPASE-PS™ (Kit) was obtained from Trinity Biotech (Jamestown, NY, USA). Ultrapure water was produced using a Millipore water purification system.

2.2. *Fruits*

Lyophilized maqui^{CHI2}, açaí^{BRZ2}, noni^{ECU} and papaya^{ECU} fruits, provided by Ecuadorian Rainforest LLC. (Belleville, NJ, USA), were obtained from Chile (CHI), Brazil (BRZ) and Ecuador (ECU). Açaí^{COL} was supplied by Corpocampo S.A. (Bogotá, Colombia (COL)). Açaí^{BRZ1} was provided by Amazon Dreams Industria e Comercio S.A. (Belem, Pará, Brazil). Cape gooseberry^{COL} fruits and calyx were provided by Arc. Eurobanan S.L. (Santa Fé de Bogotá, Colombia). Maqui^{CHI1} and maqui^{CHI3} were provided by INTA-UCHILE (Santiago, Chile): maqui^{CHI1} was lyophilized and maqui^{CHI3} was spray-dried and microencapsulated by atomization.

2.3. *Extraction*

Each sample (100 mg) was mixed with 1 mL of methanol/water (70:30, v/v). For the HPLC analysis samples were acidified with 1% of formic acid. Then, the samples were vortexed and sonicated in an ultrasonic bath for 60 min. The samples were kept at 4 °C overnight and sonicated again for 60 min. A centrifugation (model EBA 21, Hettich Zentrifugen) step (9500 *xg*, 5 min) was used to separate the supernatant from the solid residue. This supernatant was filtered through a 0.45-μm PVDF filter (Millex HV13, Millipore, Bedford, MA, USA) and stored at 4 °C before the analyses were performed.

2.4. *Identification of phenolic compounds by HPLC–DAD–ESI/MS^n and quantification by RP-HPLC–DAD*

The chromatographic analyses for the identification were carried out on a Luna C18 column (250 × 4.6 mm, 5 mm particle size; Phenomenex, Macclesfield, UK). Water/formic acid (99:1, v/v) and acetonitrile were used as the mobile phases A

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